Effects of Synthetic Versus Natural Colloid Resuscitation on Inducing Dilutional Coagulopathy and Increasing Hemorrhage in Rabbits

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Background: **On the basis of logistic benefits of colloids over crystalloids, the U.S. military selected Hextend for resuscitation of combat casualties in the field. We investigated the effects of resuscitation with this fluid, as well as other colloids, on coagulation and uncontrolled bleeding in rabbits subjected to a splenic injury.**

Methods: **Anesthetized male New Zealand white rabbits (3.3 kg ± 0.2 kg) were divided into three groups and subjected to hypothermia (35°C ± 0.5°C) and ~40% isovolemic blood exchange (hemodilution) with Hextend (H); Dextran70 (D); or 5% human albumin (A) solution (n = 8/group). Complete blood count, arterial blood gas, and coagulation values were measured before and after hemodilution. Laparotomy was performed and a standard splenic injury causing uncontrolled hemorrhage was made. Rabbits were resuscitated (25 mL/kg) with the same colloid used for hemodilution to restore baseline blood pressure. Animals were monitored for 2 hours or until death. Blood loss and survival times were measured.**

Results: **There were no differences among groups in pH, Hct, fibrinogen, or platelets before or after hemodilution. Hct, fibrinogen, and platelets were reduced by 45% to 60% in all groups. Prothrombin time (PT) and activated partial thromboplastin time were prolonged in all the rabbits with the greatest increase in A group. Thrombelastograph (TEG) analysis showed longer initial reaction (R) and clotting (K) times, slower clotting rate and lower clot strength in H and D than A diluted blood. R time was faster and K time remained unchanged in A group after hemodilution. Thrombin generation potential and peak concentration of thrombin were unchanged in A samples but significantly reduced in H and D diluted samples. Subsequent splenic injury led to almost equal blood losses (~54 ± 1 mL/kg) in H and D groups, which were higher ($p < 0.01$) than in A rabbits (37 ± 4 mL/kg). This resulted in death of 100% (H), 75% (D), and 50% (A) of the rabbits with significant difference in survival time among the groups.**

Conclusion: **TEG and thrombin generation assays identified more severe coagulopathy development with H and D than A dilution, whereas plasma PT and activated partial thromboplastin time measurements did not differentiate between these colloids. These results suggest that resuscitation with albumin maintained coagulation function, decreased blood loss, and improved survival time compared with the synthetic colloids.**

Key Words: **Hemostasis, Dilutional coagulopathy, 5% albumin, Hextend, Dextran 70, Splenic hemorrhage, Rabbit.**

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Fluid administration is one of the most basic concepts of resuscitation and is part of routine patient care in prehospital and hospital settings. However, controversy continues over the choice of the ideal fluid.[1–4] The use of colloids or crystalloids varies widely across the globe depending on personal choices, clinical experience, availability, and cost.[5] The current Advanced Trauma Life Support care guidelines call for an aggressive crystalloid resuscitation starting with 2 L of lactated Ringer's (LR) and continued bolus infusions until completion of surgical repair.[6] Although this regimen is relatively successful for early care of injured patients in the civilian sector, the Tactical Combat Casualty Care (TCCC) guidelines recommend Hextend for its logistical benefit.[7,8]

Colloid fluids have documented early advantages over isotonic crystalloids (e.g., saline). They are more efficient in expanding plasma volume than isotonic crystalloids and achieve similar hemodynamic endpoints with much smaller volumes.[9] Also, because of smaller volumes and increased oncotic pressure in plasma, colloids reduce or prevent tissue edema, unlike isotonic crystalloids which primarily fill interstitial space and can produce significant edema. Hypertonic crystalloids, on the other hand, have properties similar to colloids in plasma expansion. Low volume resuscitation with hypertonic saline restores perfusion pressure and vital signs without causing tissue edema. Although the military has a long-standing interest in 7.5% hypertonic saline, the product is not Food and Drug Administration-approved and so cannot be used. Thus, the current military TCCC (the PHTLS equivalent) guidelines call for resuscitation of combat causalities in

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shock with Hextend. Patients may be administered up to a 1 L infusion until the radial pulse can be palpated. Hextend is an artificial colloidal solution, classified as a plasma expander, and is intended to support oncotic pressure as well as provide electrolytes. It contains 6% high molecular weight (average 670 kDa) hydroxyethyl starch (hetastarch, HES) in a balanced electrolyte solution with Ca^{++}, Mg^{+}, and glucose. Hextend has been shown to be effective in normalizing organ perfusion, metabolic deficits and hemodynamic measurements of animals subjected to a controlled hemorrhage and hypotensive shock.[9,10] Hextend effects on coagulation and induced coagulopathy have been widely demonstrated in clinical and experimental studies and has been attributed to the inhibitory effect of this colloid on clotting factors,[11–15] platelet function,[16,17] and fibrin polymerization.[18,19] It is also well known that the infusion of colloids causes an efflux of all plasma proteins including coagulation factors from vascular to interstitial space reducing blood coagulation capacities.[20,21] Dextran colloids have been used as a plasma volume expander to manage hemorrhagic shock for over 50 years. Two types of dextrans (6% Dextran 70 and 10% Dextran 40) are used clinically with an average molecular weight (MW) of 70 kDa and 40 kDa. Dextrans are also known to interfere with hemostatic mechanisms, causing prolonged bleeding and clotting times, deceasing platelet count and plasma fibrinogen content.[22,23] These effects seem to be more pronounced in high MW dextran solutions.[24]

As the leading cause of potentially preventable death on the battlefield is uncontrolled truncal hemorrhage, the effect of resuscitation fluids on coagulation parameters is of utmost concern.[25] The purpose of this study was to investigate the effect of Hextend resuscitation on coagulation function and its potential impact on blood loss in a model of uncontrolled hemorrhage in coagulopathic rabbits. Hextend effects were compared with another synthetic colloid Dextran 70 and a natural colloid albumin.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research. Male New Zealand white rabbits, specific-pathogen-free, weighing 3.1 kg to 3.6 kg were used for this study. All animals received care in strict compliance with the *Guide for the Care and Use of Laboratory Animals*.[26] Before surgery, rabbits were acclimated for a minimum of 72 hours and carefully checked for preexisting diseases. After acclimation, blood samples were collected from the central ear artery and complete blood count (CBC) and standard coagulation tests [prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen] of plasma samples were measured to verify the health and normal hemostatic function of the animals. Automated clinical laboratory equipments (ABX Pentra 120 CBC Analyzer [ABX Diagnostics, Irvine, CA] and Dade-Behring BCS Coagulation Analyzer [Dade-Behring, Marburg, Germany]) were used for these hematological measurements.

On the day of surgery, blood coagulation was also evaluated with thrombelastograph (TEG) analysis using citrated arterial blood samples. The accuracy of the TEG machines (TEG Hemostasis Analyzer 5000, Hemoscope, Niles, IL) was checked daily using quality control standards obtained from Hemoscope. For this assay, clotting was initiated by adding 10 μL of human recombinant tissue factor (Innovin, diluted 1:200 with saline) and 20 μL of 0.2 mol/L $CaCl_2$ to 336 μL blood samples in the presence of a contact activation inhibitor (Corn Trypsin Inhibitor, 4.3 μL). Samples were tested in triplicate and tracing continued until 30 minutes after the clot reached maximum strength. The following variables were measured for each sample at the experimental temperatures: reaction time (R, min, the time that the initial fibrin formation is detected); clotting time (K, min, the speed of clot formation and is the time from the R time until a clot with a fixed firmness is formed); angle (α, degree, the kinetics of clot development); and maximum amplitude (MA, mm, the maximum strength or firmness of the developed clot). The velocity of clot formation was also calculated as the first derivative of the TEG tracings and maximum clotting velocity (V_{max}, mm/min) was analyzed for each sample as described previously.[27]

For thrombin generation assays, aliquots of citrated plasma samples, obtained from undiluted (baseline) and hemodiluted blood, were frozen at −70°C and assayed in batches for thrombin generation at later dates. Thrombin generation was analyzed by a fluorogenic method using a specialized plate reader and software (thrombinoscope; Synapse BV, Maastricht, The Netherlands). Thrombin generation was measured in platelet-poor plasma (80 μL) mixed with a reagent containing phospholipids and tissue factor in 96-well plates. Added to this mixture was a buffer containing a fluorogenic substrate for thrombin enzymatic activity and calcium chloride to initiate coagulation process. Generated fluorescence was measured by the spectrophotometer and data were used by the software to construct the thrombograms for determining endogenous thrombin potential (ETP), maximum concentration (peak), time to peak, and lag time for each sample. All assays were performed in triplicate.

Animal Preparation and Instrumentation

The daily food ration was withdrawn from the surgical candidates on the day of surgery while allowing free access to water. Surgical anesthesia was induced and maintained with intramuscular injections of fentanyl citrate (0.05 mg/kg), ketamine (15–25 mg/kg) and midazolam (~2 mg/kg). This regimen was further supplemented with intravenous (IV) injections of 2% methohexital (Brevital, 0.2 mL, 20 mg/kg), as needed during the laparatomy procedures. Rabbits breathed spontaneously and required no intubation. Oxygen, however, was provided at a rate of 1 L/min via a loose-fitting facemask (Pao_2 >100 mm Hg) during the experiment. Rabbit body temperature was monitored with a rectal thermocouple and maintained between 37°C and 38°C with the use of a heating pad before hemodilution.

The marginal veins in both ears were cannulated with 24G, 1.5 inches IV catheters to administer anesthetic agent and maintenance fluid (LR, 10 mL/kg/h) during surgical procedures. Resuscitation fluids were also infused through these venous lines. The right common carotid artery was also cannulated with a small (1.3 mm optimum density) gel-filled catheter attached to a precalibrated transducer (TL 11M2-D70-PCT; Data Sciences International, St. Paul, MN). Transducer measurements of blood pressure (systolic, diastolic, and mean) and heart rate were transmitted wirelessly to a receiver plate and displayed and recorded continuously by a computer system for future analysis. For the purposes of blood sampling and hemodilution, the left femoral artery of rabbits was also cannulated with an IV catheter (21 G, 2 inches length) and closed with a three-way stopcock. Arterial blood samples (baseline) were then collected from the catheter and blood gas analysis, CBC, standard coagulation tests, and TEG analysis were performed at 37°C.

Coagulopathy Induction

Twenty-four rabbits were randomly divided into three groups and subjected to a mild hypothermia and 40% isovolemic blood exchange using Hextend (H; Abbott Laboratories, Chicago, IL), Dextran 70 (D; 6% dextran 70 in saline, B. Braun Medical, Irvine, CA) or 5% human albumin (A; Baxter Healthcare, Glendale, CA) solution. Using a dual syringe pump (Harvard Apparatus, Holliston, MA), an estimated 50% of the rabbit's blood volume (35 mL/kg) was withdrawn from the femoral artery at 5 mL/min while at the same time, an equal volume of a colloid solution (25°C) was infused IV at the same rate. The 50% of the circulating blood volume was estimated based on the assumption that rabbit blood volume is equal to 7% of body weight[28] and the specific gravity of blood is equal to 1. Because blood withdrawal and colloid infusion are performed simultaneously, the actual portion of blood replaced with colloid solution is close to 40%. In addition, during blood exchange, the rabbit's core temperature was allowed to drop ~3°C below its normal level (38°C) after which it was maintained at ~35°C ± 0.5. After hemodilution and at least 10 minutes stabilization at this hypothermic temperature, arterial blood samples were collected for different assays. The standard coagulation tests of the plasma samples and the TEG assays of whole blood were performed at 35°C.

Surgical Procedure

Laparotomy was performed via a midline incision and tissue bleeding was controlled with gauze application and hemostatic clamping. After allowing a 10-minute stabilization, the baseline blood pressure was recorded. A stable mean arterial pressure (MAP) of at least 60 mm Hg was required before proceeding with the next phase of the experiment. To create the hemorrhage, a longitudinal incision (5 mm deep) was made on the spleen's dorsal capsule with a microknife as described before.[29] After the injury, the spleen was repositioned and the peritoneal cavity was rapidly closed with sutures to avoid any blood spillage. Ten minutes after the injury and hemorrhage, at which time blood pressure was substantially decreased, IV fluid resuscitation was started (1 mL/min) and targeted to raise MAP to the baseline level. The resuscitation fluid for each rabbit was the same colloid used in the earlier hemodilution procedure. To avoid excessive hemodilution, resuscitation volume was limited to 25 mL/kg, which was administered mainly during the first 30 minutes of the observation period. A small blood sample (2 mL) was collected 60 minutes postinjury for coagulation tests as described above. At the conclusion of each experiment (120 minutes postinjury or at death), survival time was recorded and surviving animals were killed by an IV injection of 1 mL pentobarbital sodium (390 mg/mL). The peritoneal cavity was then reopened; blood and blood clots were collected with dry gauze and weighed to estimate the total blood loss.

Statistical Analysis

The Tukey-Kramer and analysis of variance statistical tests were used to compare the groups for their presurgical (screening) criteria. Wilcoxon matched pair tests were used to compare the hematological changes after hemodilution with baseline values in each group. Nonparametric analysis of variance (Kruskal-Wallis test) were employed for comparison of hematological, coagulation, and blood loss measurements among the groups. Dunnett's multiple comparison test was used as the post-test to compare pairs of group means. The comparison of survival times was performed using the Log-rank test. The incidence of survival was compared using Fisher's exact test. Data are expressed as mean ± standard error. Statistical significance was assigned at greater than 95% confidence level ($p < 0.05$).

RESULTS

No significant differences in any of the baseline hematological parameters were found among the three experimental groups. Mean baseline values for all groups are shown in Tables 1 and 2. The baseline CBC, arterial blood gas, and clotting time results were within the established normal range for adult Male New Zealand white rabbits at our institute.

Coagulopathy Induction

After instrumentation (before hemodilution), a mild respiratory acidosis (pH 7.32) was measured in anesthetized rabbits with elevated $Paco_2$ levels (56.5 ± 4.1 mm Hg). This is a common occurrence in spontaneously breathing anesthetized rabbits.[22] The use of an oxygen mask, however, maintained the arterial Pao_2 level above 100 mm Hg in all the subjects.

The hypothermic and dilutional coagulopathy reduced hematocrit, red blood corpuscles, and platelet counts of blood equally irrespective of the type of colloid used (Table 1). Blood pressure was best maintained during blood exchange

Table 1 **Cell Counts and Physiological Measurements Before (Baseline) and After Hemodilution With Different Colloids**

Measured Parameters	Baseline[+]	Hextend Hemodilution	Dextran 70 Hemodilution	Albumin Hemodilution
RBC (10^5/mm^3)	5.7 ± 0.1	2.7 ± 0.1*	2.2 ± 0.1*	2.3 ± 0.2*
Hct (%)	35.7 ± 0.5	16.7 ± 0.5*	13.5 ± 0.4*	14.7 ± 0.9*
Platelet (10^3/mm^3)	352 ± 22.5	160 ± 13*	241 ± 24*	195 ± 21*
MAP (mm/Hg)	88.7 ± 1.8	79.9 ± 2.8*	88.3 ± 2.4†	76.8 ± 5.4*
pH	7.32 ± 0.01	7.4 ± 0.04	7.4 ± 0.02	7.35 ± 0.01
Base excess (mM)	0.1 ± 0.6	0.6 ± 0.7	−0.5 ± 1.6	−0.14 ± 1.0
Body temp. (°C)	37.4 ± 0.1	35.1 ± 0.2*	34.8 ± 0.3*	35.5 ± 0.1*
Ca^{++} (mM)	1.55 ± 0.02	1.45 ± 0.03	1.4 ± 0.03	1.3 ± 0.04*

Hct, Hematocrit; MAP, mean arterial pressure; Ca^{++}, Ionized calcium.
[+] There were no differences among groups; values represent the average of the three groups.
* $P < 0.01$ compared to corresponding baseline (Wilcoxon matched pairs test).
† $P < 0.05$ compared to Albumin hemodilution (Dunnett's multiple comparison test).

Table 2 **Standard Coagulation and TEG Measurements of Rabbit Blood Before (Baseline) and After Hemodilution With Different Colloids**

Coag/TEG Parameters	Baseline[+]	Hextend Hemodilution	Dextran 70 Hemodilution	Albumin Hemodilution
PT (seconds)	11.1 ± 0.1	13.0 ± 0.3*	13.6 ± 0.2*	15.4 ± 0.7*‡
aPTT (seconds)	17.7 ± 0.4	35.5 ± 7.8*	44.6 ± 4.6*	53.5 ± 7.2*
Fibrinogen (mg/dL)	172.1 ± 9.3	92.7 ± 9.8*	83.0 ± 7.3*	81.7 ± 7.8*
R (min)	4.5 ± 0.1	4.6 ± 0.7	4.9 ± 0.4	3.8 ± 0.5*†
K (min)	1.7 ± .05	4.4 ± 1.6*	3.5 ± 0.7*	1.9 ± 0.4£
α angle (°)	67.5 ± 0.6	44.8 ± 7.2*	49.1 ± 4.2*	64.5 ± 3.6£
MA (mm)	65.0 ± 0.8	36.3 ± 4.1*	39.9 ± 2.6*	49.0 ± 5.5*£
Vmax (mm/min)	14.5 ± 0.4	5.7 ± 1.2*	6.5 ± 1.0*	11.4 ± 1.8£

[+] There were no differences among groups; values represent the average of the three groups.
* $P < 0.01$ compared to corresponding baseline (Wilcoxon matched pair test).
‡ $P < 0.05$ vs. Hextend only (Dunnett's multiple comparison test).
† $P < 0.01$ vs. Dextran only (Dunnett's multiple comparison test).
£ $P < 0.01$ vs. Dextran or Hextend group (Dunnett's multiple comparison test).

with Dextran 70, which may suggest greater oncotic effect of Dextran over the other colloids. The MAP of rabbits infused with Hextend or Albumin decreased by approximately 10 mm Hg after hemodilution, while the MAP of animals infused with Dextran 70 was similar to baseline (Table 1). The average MAP of each group during hemodilution and subsequent hemorrhage and resuscitation is shown in Figure 1. MAP decreased in rabbits infused with albumin during blood exchange, but recovered to near baseline levels at the end of the infusion. At the time of injury, there was no difference in the MAP of rabbits hemodiluted with albumin (71 ± 6 mm Hg) or Hextend (76 ± 3 mm Hg). However, the MAP of the Dextran group (81 ± 7 mm Hg) was higher ($p < 0.05$) than the albumin-infused animals.

Despite the minor differences in blood pressure, hypovolemia and tissue hypoperfusion were likely avoided in all the rabbits as indicated by normal pH and arterial base excess levels of the blood samples collected after hemodilution (Table 1). The rabbits' body temperatures were reduced by 2.5° to 3.0°C with no differences among the groups (Table 1). The ionized calcium concentration was 16% lower in albumin-infused rabbits compared with their baseline values ($p <$ 0.05). No other differences were found in arterial blood gas measurements among the groups' posthemodilution.

As expected, the changes in coagulation parameters after hemodilution were indicative of dilutional coagulopathy (Table 2). The PT was significantly prolonged in all groups compared with baseline, with a greater increase in the albumin group than in the Hextend group ($p < 0.05$). The changes in aPTT among the groups were more pronounced than in PT measurements. The aPTT was prolonged 2 to 3 times of the baseline values, and again, albumin infusion appeared to have the greatest impact. PT and aPTT were measured at 35°C, the rabbit body temperature after hemodilution. Plasma fibrinogen was equally reduced (~50%) in all three groups. The TEG analysis (Fig. 2) also indicated hypocoagulability, but effects of the individual colloids were different from those measured by PT and aPTT (Table 2). The *R* time was not changed by Hextend or Dextran but was significantly shortened with albumin infusion. The clotting time (*K*) was prolonged with Hextend and Dextran, but did not change from baseline with albumin use. Also, there was a significant decrease in clot formation rate (α angle) with synthetic colloids, which was not seen in albumin-diluted

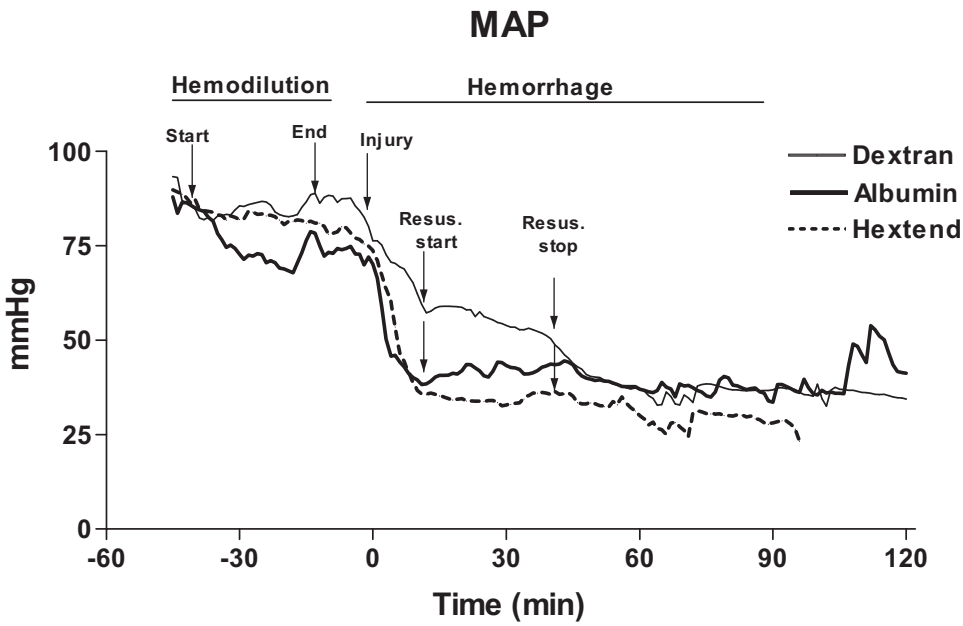

Fig. 1. *Averages of mean arterial pressure (*MAP*) of rabbits resuscitated with different colloids throughout the experiment. The MAP of Dextran-infused animals did not change during hemodilution and remained significantly higher (*$p < 0.05$*) before and 10 minutes after the splenic injury when compared with albumin or Hextend group. There was no difference in MAP of rabbits at 60 minutes postinjury.*

blood. The maximum clotting velocity (V_{max}) followed a similar pattern. The maximum clot strength (MA) was significantly reduced in all three groups, but the decreases were more pronounced with synthetic colloids than albumin.

Thrombin generation measurements (Table 3) showed that Lag time which corresponds to *R*-time in TEG, was shortened by albumin dilution but was not significantly changed by the other colloids. Total thrombin generation (ETP) and maximum thrombin concentration (peak) reached after addition of clotting agents were unchanged in albumin diluted samples but reduced significantly in the plasma samples diluted with synthetic colloids. Time to peak concentration was only shortened in the albumin group compared with baseline. The average thrombograms for each hemodilution and the undiluted blood (baseline) are shown in Figure 3.

Bleeding Outcome

The splenic injury produced a profuse hemorrhage, causing a rapid decrease in MAP (~35 mm Hg) in all coagulopathic rabbits. Ten minutes after injury (before resuscitation), the MAP of Dextran-treated rabbits was significantly higher than the other two groups (Fig. 1). Rabbits were resuscitated (25 mL/kg) with the same fluid as used for hemodilution for approximately 30 minutes during which the blood pressure did not significantly increase and bleeding continued. At 60 minute postinjury, the PT was further prolonged with no difference among groups (20.9 seconds ± 1.7 seconds), and fibrinogen and aPTT could not be measured because of extreme dilution of the blood samples (no clot was formed).

At the conclusion of the experiment (120 minutes postinjury), 50% of albumin (4/8), 25% of Dextran 70 (2/8), and 0% of Hextend (0/8) infused rabbits survived the hemorrhage. Nonsurviving rabbits exsanguinated between 53 minutes and 110 minutes postinjury. Total blood loss was significantly less in the albumin-treated group compared with other colloid-treated animals ($p < 0.05$; Fig. 4). Kaplan-Meier analysis of survival time also indicated significant differences ($p = 0.0004$) among the three groups: albumin resuscitation was associated with the longest survival time with no difference between the synthetic colloids (Fig. 5).

DISCUSSION

This study investigated the effect of Hextend resuscitation, as compared with two other colloids, on coagulation properties and bleeding outcome in rabbits subjected to an uncontrolled bleeding injury. The hemorrhage model and the outcome of coagulopathic bleeding and shock in rabbits were detailed in a previous report.[29] In this study, resuscitation with Hextend and the other colloids produced similar degree of hypothermia and dilutional coagulopathy in the rabbits as evidenced by equal reduction in body temperature, hematocrit, platelets, and fibrinogen concentration postinfusion. However, despite these similarities, the changes in coagulation, as measured by standard coagulation tests, TEG and thrombin generation assay, varied significantly with respect to each colloid. The prolonged PT and aPTT test results suggested that a more severe coagulopathy was produced with albumin infusion, whereas the TEG and thrombin generation analysis indicated greater coagulation impairment with the synthetic colloids. The shorter *R* time (TEG analysis) and shorter lag time (thrombin generation assay) in albumin

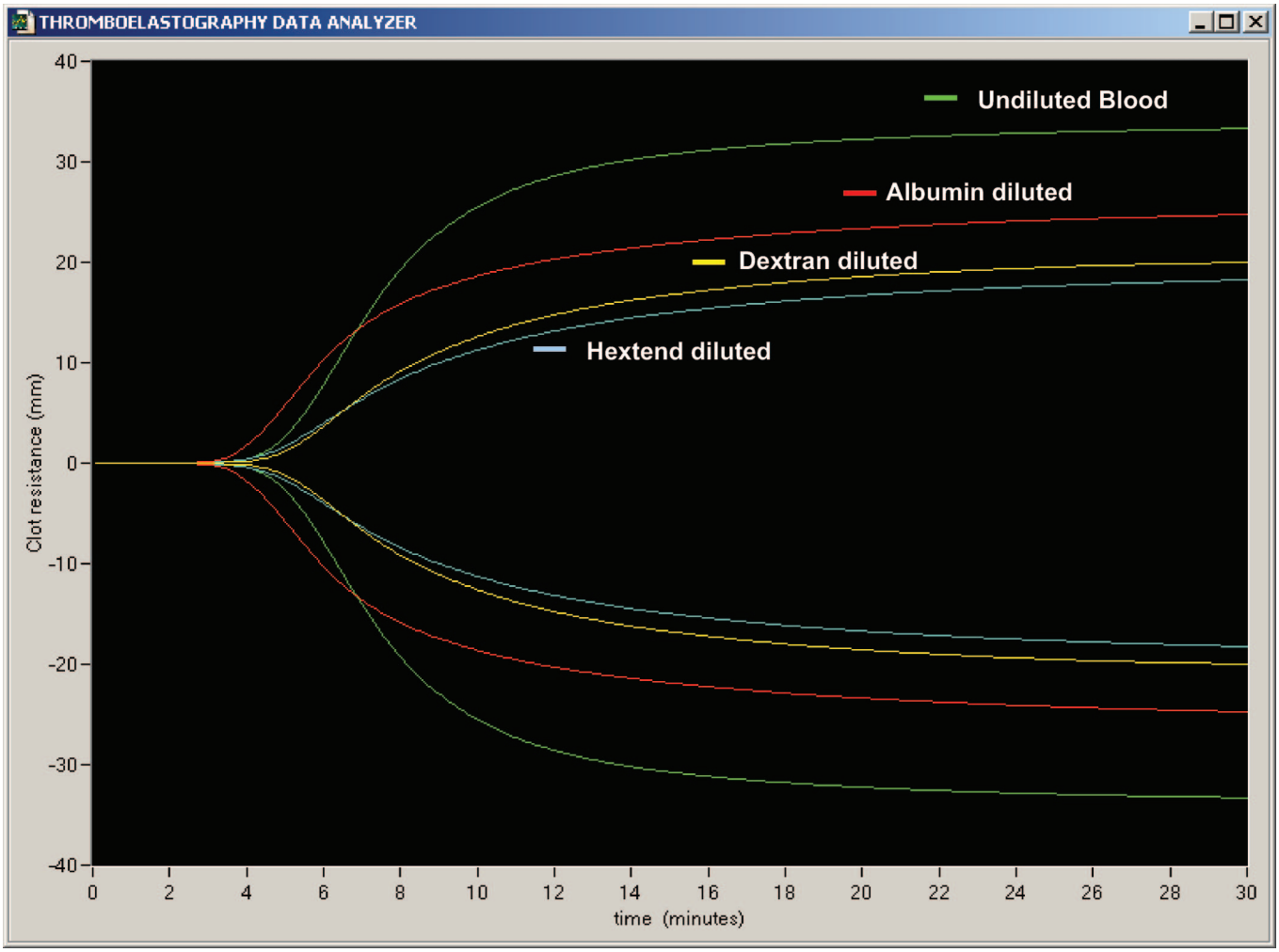

Fig. 2. *Averages of the TEG tracings of rabbit blood before (undiluted) and after hemodilution with different colloids.*

Table 3 Thrombin Generation Measurement of Plasma Before (Baseline) and After Hemodilution With Different Colloids

Thrombogram Parameters	Baseline[+]	Hextend Hemodilution	Dextran 70 Hemodilution	Albumin Hemodilution
Lag time (min)	2.8 ± 0.1	2.6 ± 0.1	3.1 ± 0.2	2.2 ± 0.1*†
ETP (nM × min)	405 ± 31.1	331 ± 5.0*	319 ± 4.5*	443 ± 5.6
Peak (nM)	113.6 ± 10.9	64.5 ± 1.8*	63.1 ± 0.8*	118.6 ± 1.4
tt-Peak (min)	5.5 ± 0.2	5.7 ± 0.1	6.2 ± 0.1	4.4 ± 003*

Lag time, time until thrombin is detected; ETP, endogenous thrombin potential; Peak, maximum thrombin concentration, tt-peak, time to reach peak.

[+] There were no differences among groups; values represent the average of the three groups.

* $P < 0.05$ compared to corresponding baseline (Wilcoxon matched pair test).

† $P < 0.01$ vs. Dextran only (Dunnett's multiple comparison test).

resuscitated animals suggested hypercoagulation, which may be caused by dilution of antithrombin activity in rabbit plasma, as reported elsewhere.[30] In contrast, hemodilution with Hextend and Dextran resulted in hypocoagulation as evidenced by the decrease in ETP, thrombin peak, clotting rate, and more pronounced reduction in clot strength with no changes in R and lag times in the samples. The reduced activities of vitamin K-dependant coagulation factors (factor II, VII, IX, and X) following Hextend hemodilution, which corresponded to increased bleeding and high mortality, were reported in our previous rabbit study.[29] The subsequent injury and uncontrolled hemorrhage confirmed the accuracy of TEG and thrombin generation measurements in diagnosing the severity of coagulopathy produced with each fluid. The greater sensitivity of TEG measurements as better indicators of coagulopathic bleeding and mortality compared with stan-

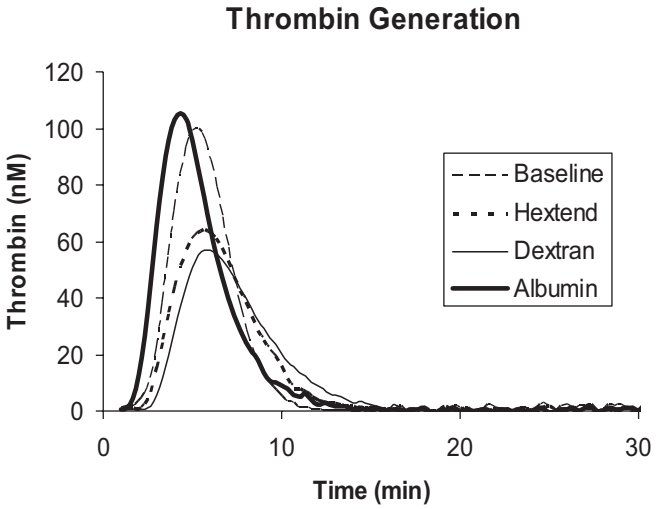

Fig. 3. *Averages of the thrombogram traces for each group of plasma samples before (*baseline*) and after in vivo hemodilution with different colloids.*

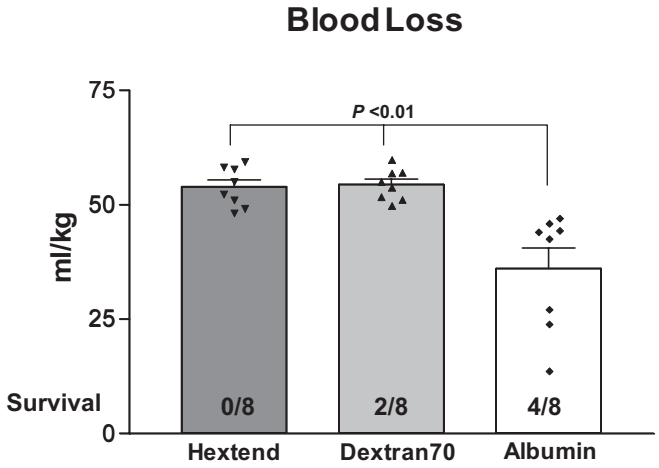

Fig. 4. *Averages of total blood loss of hemodiluted rabbits following splenic injury and resuscitation with different resuscitation colloids. The* square *and* triangle *symbols represent the bleeding volume of the individual rabbits. The survival rates, 0/8, 2/8, and 4/8 were not statistically different.*

dard clotting tests (PT, aPTT) was also demonstrated in our earlier study in this rabbit hemorrhage model.[29] In a similar rabbit study, the coagulation changes (TEG analysis), antithrombin activity and factor VIII complex activity were analyzed after isovolemic hemodilution (40%) with Hextend or 5% albumin without inflicting any injury or hemorrhage.[30] The antithrombin activity was reduced equally in both colloid groups but Factor VIII complex activity was significantly reduced only in Hextend-treated animals. This resulted in a hypercoagulable state (shorten *R*-time and increased α) that was evident only in the albumin diluted group.[30]

Our TEG data are consistent with several in vitro studies in which blood was diluted with hydroxyethyl starch, gelatin, albumin or dextran, and coagulation changes were measured.[31–33]

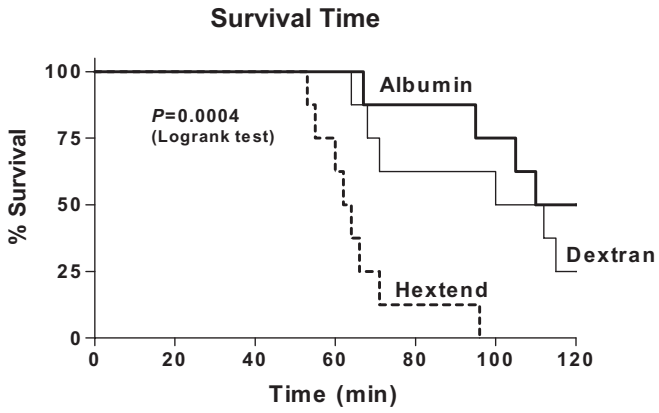

Fig. 5. *The Kaplan-Meier analysis of survival time of different rabbit groups. Albumin-treated rabbits lived longer than the other two groups with no difference between Dextran- and Hextend-treated groups.*

Our study also allowed correlating the coagulation changes measured after in vivo hemodilution to the outcome of subsequent uncontrolled hemorrhage which verified the diagnostic value of each test in predicting increased coagulopathic bleeding. We recognize that the differences in blood loss and survival times among the three colloid groups were likely caused by excessive administration of these fluids to the animals, which were above the recommended dosage. This was the main limitation of this study. However, in cases where differences cannot be easily measured, extreme testing conditions are required to differentiate among products that have similar main properties (e.g., plasma expansion), but substantially different side effects. Coagulation impairment and increased risk of spontaneous bleeding in patients who received high MW HES colloid even with limited volume have been reported.[34–37]

Colloids are clearly more efficient than isotonic crystalloids in attaining resuscitation endpoints at least acutely, requiring smaller fluid volumes. This clear logistic advantage of colloids over isotonic crystalloids has prompted the selection of colloids for prehospital treatment of hemorrhagic shock in the U.S. military. Hypertonic saline (7.5%), which is also an efficient small-volume plasma expander, was not considered for this purpose because this fluid is not approved by the Food and Drug Administration for human use and the military is prohibited from using it by DoD policy. Hespan was originally recommended in a 1996 Tactical Combat Casualty Care paper as a better alternative for fluid resuscitation in the field than crystalloid (LR) solution.[38] Like Hextend, Hespan is a synthetic colloid which contains 6% HES colloid, but is dissolved in normal saline. Earlier studies of Hespan and albumin in surgical patients showed no difference in blood loss between the two colloids.[39,40] However, increased bleeding was reported in more recent studies in patients undergoing open heart surgery with cardiopulmonary bypass after the use of Hespan.[41,42] Hespan was later replaced in the TCCC guideline with Hextend, a 6% HES prepared in a balanced electrolyte, lactated buffer solution with glucose.

The HES in Hextend and Hespan has an average of 670 kDa MW with 0.75 degree of hydroxyethyl substitution (~75 hydroxyethyl group attachment per 100 glucose units, 670/0.7). Hextend was shown to be safer than Hespan, causing less blood loss and required less calcium supplementation in surgical patients treated for hypovolemia.[43] A protective influence of Hextend against multiple organ failure attributable to a potential antioxidant effect of the HES molecule was also reported.[44] This effect is not unique to HES as albumin and other fluids such as hypertonic saline without and with dextran also have reported antioxidant properties.[44] However, the inhibitory effects of Hextend and Hespan on coagulation and platelet function, as measured by TEG analysis, were similar for both fluids.[45] It should be noted that other formulations of HES colloids with smaller MW and less degree of substitution are available and used preferably in other countries. Evidence from in vitro experiments[46–48] and clinical studies[49–52] indicate that the HES colloids with smaller MW and less hydroxyethyl substitution appear to be safer, more rapidly metabolized, and cause less coagulation impairment than the larger MW with higher hydroxyethyl substitution solutions.

The mechanism of coagulopathy induced by infusion of HES is multifactorial. It causes a reduction in plasma concentration of coagulation factor VIII[11–13] and von Willerbrand factor (vWF),[14,15] inhibition of platelet function,[16,17] and decreased interaction of activated factor XIII with fibrin polymer.[18,19] The latter effect causes slowly growing and weaker clot formation which are subject to faster fibrinolysis.[53] The inhibition of platelet function is caused by a decrease in expression or blocking of platelet fibrinogen receptor glycoprotein IIb–IIIa.[54,55] In addition to these effects, the oncotic force of synthetic or natural colloids also have a significant influence on coagulation. Infusion of colloids causes efflux of plasma proteins from blood to interstitial space that is driven by the oncotic forces. In an experimental hemorrhage study,[20] resuscitation with Hespan-supplemented fluid lowered all serum proteins including albumin, globulin, and coagulation proteins (fibrinogen and factor VIII) and concomitantly increased these proteins in lymphatic vessels. The significant reduction in fibrinogen and factor VIII activity persisted throughout the postresuscitation period in animals.[20]

Among synthetic colloids, dextran 40 is the most powerful inhibitor of coagulation and has been used clinically for prevention postoperative venous thrombosis and pulmonary embolism for years.[34] In vitro 50% hemodilution of patient blood with 10% dextran 40 colloid completely inhibited clot formation in TEG assays.[32] Dextran 70 has lesser effects on coagulation than Dextran 40 and is clinically used as plasma volume expander for treating hypovolemia. The inhibitory effects of Dextran on coagulation have been attributed to binding to fibrinogen and interfering with fibrin cross-linking process, reducing factor V, VII, and VIII concentrations in plasma and inhibition of platelet aggregation.[22,23,56] Our results showed no significant difference in coagulation effects of Hextend and Dextran 70 in rabbits.

Albumin resuscitation in this current study resulted in a decrease in ionized calcium concentration in blood. This change may be because of binding of albumin to ionized calcium and was reported in albumin-resuscitated shock patients previously.[57] Similar to synthetic colloids, the oncotic pressure of albumin also causes the efflux of plasma protein to interstitial fluid space that may affect coagulation.[21] In a randomized prospective study, patients who received albumin-supplemented resuscitation showed significant prolongation in prothrombin time and reduced fibrinogen and prothrombin protein levels compared with crystalloid resuscitated patients.[58] In general, however, albumin offers several advantages compared with synthetic colloids, including less restrictive dose limitations, lower risk of coagulation impairment, absence of tissue deposition leading to pruritus, reduced incidence of anaphylactic reactions, and ease of monitoring to prevent fluid overload.[59] Albumin also increases oxygen delivery significantly more than LR and improves organ microcirculation.[60] A meta-analysis of randomized controlled trials comparing albumin and hydroxyethyl starch (HES) solutions in cardiopulmonary bypass patients concluded that the postoperative blood loss was significantly lower in the patients infused with albumin than HES fluids.[37] In 1998, two systematic reviews (meta-analysis) of many clinical trials concluded that resuscitation with albumin was associated with higher risk of mortality than with crystalloids.[2,61] A more recent (2001) and comprehensive meta-analysis by Wilkes and Navickis[62] demonstrated a trend toward increased mortality when albumin is used to resuscitate surgical and trauma patients, a trend that did not reach statistical significance. A 2004 multicenter clinical trial in Australia comparing the effect of 4% albumin with saline in intensive care unit patients found no difference in mortality and other outcomes between the two fluids.[63] However, analysis of data for critically ill patients with traumatic brain injury showed higher mortality to be associated with fluid resuscitation with albumin than saline.[64] These reports taken together with other known disadvantages of albumin such as its critical short supply and high cost, as compared with crystalloids, lessen our enthusiasm for albumin use for prehospitals resuscitation.

The ideal fluid for resuscitation of combat casualties remains elusive. It was described that the optimal fluid for the resuscitation of the hemorrhaging battlefield casualty should provide rapid plasma volume expansion preventing or treating hypovolemic shock. It should supply oxygen and other metabolic needs to hypoxic cells. Also, given the unique logistical, tactical, and medical constraints of the far-forward military environment, it is imperative that the required volume (overall weight) of fluid be minimal.[65] This requirement led to a significant amount of research by the U.S. Army into hypertonic/hyperoncotic fluids.[66] Other important criteria that should be added to this list are that the fluid should not impair coagulation function and preferably could restore normal coagulation proteins and minimize inflammatory responses. The results of this study suggest that the use of high MW synthetic colloids should be minimized or preferably be

replaced with other low-volume resuscitation fluids (crystalloid or colloid) to reduce the risk of increased bleeding and complications associated with uncontrolled bleeding in combat casualties.

In summary, this study investigated the effect of Hextend resuscitation, as compared with two other colloids, on coagulation properties and bleeding outcome in rabbits subjected to an uncontrolled bleeding injury. The coagulation impairment by the synthetic colloids, Hextend and Dextran 70, were comparable, reducing thrombin generation, clot formation rate, and strength of clot. Albumin, however, did not affect thrombin generation, enhanced the initial clotting reaction and reduced clot strength modestly. These differences in coagulation led to increased blood loss and shorter survival time in rabbits infused with artificial colloids than in those receiving albumin. TEG and thrombin generation assays accurately analyzed the extent of coagulopathy by each colloid which correlated with the subsequent bleeding after the injury. On the other hand, standard coagulation tests (PT, aPTT, and fibrinogen) did not differentiate among the colloids and the small changes in PT did not suggest significant coagulopathy with synthetic colloids. In view of these results and the overwhelming evidence in the literature regarding coagulopathic effect of Hextend, the use of this colloid for resuscitation must be balanced between its logistical advantages and impairment of coagulation function. It should be noted that albumin was included in the present study for comparison purposes as a control group and we are not advocating replacing Hextend with albumin for resuscitation of combat casualties based on this investigation. As with any therapeutic strategy, the benefits of use must be weighed against the risks. The present study clearly shows that despite published reports on the early hemodynamic benefits and volume sparing effects of colloids, some colloids have detrimental effects on coagulation and their use can certainly be problematic in an actively bleeding patient. Therefore, the search for alternative small volume crystalloids or colloids with minimal adverse effect on coagulation must be continued for safer and more effective resuscitation of combat casualties on the battlefield.

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DISCUSSION

Dr. Charles E. Lucas (Detroit, Michigan): Those who do not read the trials and tribulations of history are doomed to relive them. More than 50 years ago Dr. Francis Moore wondered why the administration of an artificial colloid reduced the coagulation proteins and promoted bleeding.

More than 25 years ago Lucas and Ledgerwood answered his question, showing that the resultant rise in intravascular oncotic pressure caused an extra vascular relocation of all serum proteins, both in man and animal studies.

This efflux into the interstitial space was directly related to the intravascular oncotic pressure. My first question, therefore, is have you measured or calculated the intravascular oncotic pressure in your three experimental colloid supplemented groups?

Force relocation of serum proteins is related to hydrostatic pressure, ionic change, and molecular weight or size. Thus, the reduction in factor VII, the smallest coagulation protein, is greater than fibrinogen and factor VIII, the larger coagulation proteins.

We show that a reduction in one or more of the coagulation factors below 25 percent of normal activity was associated with coagulopathy as judged clinically and in the test tube.

My second question is have you monitored any of the coagulation factor activities in order to more accurately define your coagulation abnormalities?

The TEG, thrombin time, PTT are all wonderful tests but we really need to know the specific coagulation factor activity of those factors which are leading to a clot formation.

The detrimental effects of colloid resuscitation on coagulation were summarized in the last Scutter Oration of the 20th Century and published in the first *Journal of the American College of Surgeons* in the first issue of the 21st Century.

These detrimental effects were also presented in the 21st Century Natal Symposium on resuscitation and later published in the 2003 issue of the *Journal of Trauma*.

Therefore, my next question is, who were the important people on the committee referred to in your manuscript who recommended that my government resuscitate my fellow citizens with a solution known to be coagulopathic?

Why not provide field resuscitation with lightweight, low-volume hypertonic saline followed by palan salt solution, blood and blood products when the patient is evacuated?

The last Scutter Oration of the 20th Century emphasized that the medical industrial complex would continue to promulgate expensive colloid solutions which are more profitable.

I mistakenly added that the erudite trauma surgeons of America would insist that colloid resuscitation studies in animals assess total protein fluxes rather than just short-term intravascular changes. How wrong I was.

My friends, we are responsible for insisting that the leaders of this organization appoint members to the Program Committee who will insist that all colloid resuscitated abstracts in animal models identify resultant interstitial fluid exchanges as monitored by pre-nodal and post-nodal lymphatic protein changes.

Only then will the AAST be truly a leader in the 21st Century. Thank you for your attention.

Dr. Mark G. McKenney (Miami, Florida): In our laboratory Dr. Ken Proctor at Miami is very interested in this subject, also. And I was interested in the actual protocol.

It appears to me that you resuscitated with a set volume instead of to a set resuscitation. Usually in resuscitation bays we resuscitate until we get the appropriate blood pressure.

So my question is, do you think you would have had a different outcome if you resuscitated not to the set volume but to a set blood pressure?

Dr. John B. Holcomb (San Antonio, Texas): I'm going to interject at this point and take the privilege of the podium. Dr. Lucas, I am now speaking as one of the senior representatives of the Committee on Tactical Combat Casualty Care, a committee of Army, Navy, and Air Force physicians and medics, representing all levels of care. Sir, I believe you attended a meeting at USUHS in 2001, whose proceedings were published in the *Journal of Trauma* in 2003, and this exact issue was extensively discussed. The products that I think you're recommending are not FDA approved and so we have a little problem there. Namely, we, the DOD, can't use them. The products we use on the battlefield have to be FDA approved.

In addition to satisfying the scientific requirements, the proposed fluids have to meet strict logistical limitations. To have a fluid widely available on the battlefield and in the hands of medics you have to have small volume fluids. Clearly, there are other fluids that are likely better but balancing all the requirements, when you weigh everything together, the decision made in 2001 was to utilize an FDA approved, small volume fluid.

I think we would all recognize there is no one perfect fluid. The primary, overriding concern is that by law, whatever is chosen has to have FDA approval.

Dr. Bijan S. Kheirabadi (Fort Sam Houston, Texas): Thank you, Dr. Lucas, for insightful review of manuscript and the comments. Unfortunately, we did not measure oncotic pressure, intravascular oncotic pressure in our plasma samples. However, we will attempt to see if there is some plasma left over we can look at that aspect.

Secondly, with respect of coagulation factor, although we didn't measure it in these studies, but in previous studies which we also used Hextend for hemodilution, we did measure it and the significant reduction we found was in factor VII activities in that group, which caused that, produces that hemodynamic coagulopathy, dilution of coagulopathy.

Who were the people who made that decision? It is way beyond my power. It is way beyond me. Maybe some of the authority in this room can tell you who are the people who made that decision.

In fact, that was one of the things that I have come across, that looking at the last literature in the past ten years or so it is a clear evidence that Hextend is one of the most coagulopathic solutions and could have been selected.

Alternative solution of lower molecule weight are, seem to have less effect on coagulation and being safer and degradate much faster and metabolize. Why they were not selected is part of it because they are not FDA approved. Should there be an effort to get some other fluid onboard and use those? Absolutely.

In terms of a question raised about why the resuscitation was targeted to the volume rather than the pressure, in fact we did target it to our pressure to raise the pressure to mean, baseline mean arterial pressure.

However, since these animals have active bleeding the pressure did not rise and we basically came to a conclusion we cannot continue resuscitating for all the two hours, but limit it after giving 25 ml per kilogram resuscitation was stopped because there was no advantage seeing it. It just would have caused more and more hemodilution and the animal will die from hypoxia.

So, therefore, again, the resuscitation target was bringing the pressure up but, as you saw, there was no changes and therefore all the animal get equal volume, that restricted volume that we predetermined.